Rapid assessments for the pancake batter tunicate, *Didemnum vexillum* (Kott 2012) in southwest New Brunswick (October 2012) and southwest Nova Scotia (September 2013)

Dawn Sephton and Bénédikte Vercaemer

Science Branch
Coastal Ecosystem Science Division

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ABSTRACT

Sephton D., and Vercaemer, B. 2015. Rapid assessment for the pancake batter tunicate, *Didemnum vexillum* (Kott 2002) in southwest New Brunswick (October 2012) and southwest Nova Scotia (September 2013). Can. Tech. Rep. Fish. Aquat. Sci.: iii + 33 p.

Rapid assessment surveys were conducted in the coastal waters of two regions of Atlantic Canada "at risk" for the arrival and establishment of *Didemnum vexillum*. In October 2012, a rapid assessment was conducted in Passamaquoddy Bay and the Western Isles region of the Bay of Fundy (BoF), close to Eastport, Maine (ME), where the species has been present since 2003. Despite the existence of natural and anthropogenic vectors of introduction and spread (tidal and current exchange, commercial and recreational vessel traffic, scallop fishing and aquaculture) in this region, *D. vexillum* was not detected. A second rapid assessment was conducted on the southwest shore of Nova Scotia between Yarmouth and Shelburne in September 2013. While this area had been the site of introduction of other non-indigenous tunicates, due to the high volume of shipping and recreational boating arrivals from the US and commercial fishing activities, *D. vexillum* was not detected here either. Monitoring for this noted global invader, which rapidly establishes in coastal, rocky benthic habitats of the type abundant in these two regions, should continue, especially in light of its discovery in the upper Minas Basin of the BoF in 2013.

RÉSUMÉ

Sephton, D., and Vercaemer, B. 2015. Évaluation rapide du tunicier pâte à crêpes, *Didemnum vexillum* (Kott 2002) au sud-ouest du Nouveau-Brunswick (Octobre 2012) et au sud-ouest de la Nouvelle-Écosse (Septembre 2013). Can. Tech. Rep. Fish. Aquat. Sci.: iii + 33 p.

Des évaluations rapides ont été menées dans les eaux côtières de deux régions du Canada Atlantique «à risque» pour l'arrivée et l'établissement de *Didemnum vexillum*. En Octobre 2012, une évaluation rapide a été menée dans la baie Passamaquoddy et la région des Western Isles de la baie de Fundy (BdF), près de Eastport, Maine (ME), où l'espèce est présente depuis 2003. Malgré l'existence de vecteurs naturels et anthropomorphiques d'introduction et de propagation (marées et courants importants, navigation commerciale et de plaisance, pêche du pétoncle et aquaculture) dans cette région, *D. vexillum* n'a pas été détecté. Une deuxième évaluation rapide a été menée sur la rive sud-ouest de la Nouvelle-Écosse entre Yarmouth et Shelburne en Septembre 2013. Bien que cette région corresponde à un des sites d'introduction d'autres tuniciers non indigènes, en raison probablement du volume élevé du transport maritime et de la navigation de plaisance en provenance des États-Unis, et des activités de pêche commerciale, *D. vexillum* n'a pas non plus été détecté. La surveillance de cet envahisseur mondial noté, qui peut s'établir rapidement dans les habitats benthiques rocheux côtiers, typiquement abondants dans ces deux régions, devrait se poursuivre, en particulier au vu de sa découverte dans le bassin Minas de la BdF en 2013.

1.0 Introduction

Aquatic invasive species (AIS) pose significant risk to biodiversity and ecosystem function on a global scale (Sala et al. 2000; Herborg et al. 2009), in addition to their negative economic impacts (Pimentel et al. 2000). The colonial ascidian *Didemnum vexillum* (Kott 2002) is a notorious rapid global invader (Lambert 2007, 2009), which colonizes artificial and natural hard substrates. Commonly known as the pancake batter tunicate, this species exhibits several traits which enhance its ability to spread and colonize rocky, benthic habitat, namely: high growth rates (Daniel and Therriault, 2007), ability to spread by fragmentation and re-attachment (Bullard et al. 2007b, Carmen et al. 2014; Morris and Carman 2012) and wide ranging environmental tolerances (Daniel and Therriault, 2007). It may overgrow benthic organisms such as bivalves (Valentine et al. 2007 a, b) and has the ability to alter the abundance (Dijkstra et al. 2007a) and biodiversity of marine fouling communities (Dijkstra et al. 2007b), Consequently, it has the potential to have significant negative impacts on shellfish harvesting and aquaculture and native benthic communities (Valentine et al. 2007a; Herborg et al. 2009). Originally from the northwest Pacific ocean (Stefaniak et al. 2012), this species has been found in temperate waters off New Zealand (Lambert 2009), Britain (Griffith et al. 2009), both coasts of the United States (US) (Bullard et al. 2007a; Valentine et al. 2007a; Lambert 2009) and the Pacific coast of Canada (Daniel and Therriault 2007), with new invasions noted recently in Ireland (Minchin and Sides 2006), Alaska (Cohen et al. 2011), and the Mediterranean (Tagliapietra et al. 2012).

Didemnum vexillum has likely been present on the Atlantic coast of the US since the early 1970's (Dijkstra et al. 2007a), but was reported initially in the Damariscotta River, Maine (ME) in 1993 (Valentine 2003, Cohen 2005). In 2002, it was discovered on rocky bottom on the scallop grounds on the US side of George's Bank (Bullard et al 2007a; Valentine et al 2007b). It is now well established at many locations from New York as far north as Eastport, Maine (ME) (Martin et al. 2011), in the Western Isles Region of the Bay of Fundy (BoF) and less than 2 km from Canadian waters, as well as in the Minas Basin, off Digby and Yarmouth in Nova Scotia (Vercaemer et al. 2015). It has been identified as a "high-risk" invader to Atlantic Canadian waters of the BoF and the southwest and south shores of Nova Scotia (NS) (Therriault and Herborg 2007). Considerable, potential suitable habitats exist in this region, in addition to the existence of multiple anthropogenic vectors of introduction and spread from established populations in nearby US waters.

Given the impact that other invasive tunicates, namely *Styela clava* and *Ciona intestinalis*, have had on shellfish aquaculture in Atlantic Canada since the mid-1990's (Carver et al. 2003; Ramsay et al. 2008) and the danger posed to native benthic communities by other potential tunicate invaders (Locke 2009), Fisheries and Oceans

Canada (DFO) implemented an AIS Bioufouling Monitoring (BFM) and Early Detection Program in 2006. In particular, this program has deployed monitoring plates annually in the New Brunswick (NB) portion of the Bay of Fundy (LeGresley et al. 2008, Martin et al. 2011) and along the Atlantic coast of Nova Scotia (Sephton et al. 2011, 2014, 2015) to facilitate the detection of a suite of non-indigenous tunicate species. Annual biofouling monitoring of 18-21 stations in southwest NB (Martin et al. 2011) and 50-75 stations along the southwest and south shores of NS between 2006 and 2014 (Sephton et al. 2011, 2014, 2015) did not detect *D. vexillum*.

The first annual Rapid Assessment survey, modeled on early-detection surveys conducted on the eastern US coast (Pederson et al. 2005), was conducted in southwest NB in Passamaquoddy Bay and the Western Isles Region of the BoF in 2009 (Martin et al. 2010). The goal of this survey was to use additional detection techniques, such as collecting benthic dredge samples, snorkeling and scuba diving surveys, to search for *D. vexillum*. Although a native didemnid species, *D. albidum*, was detected during the initial survey, and subsequent surveys in 2010 and 2011 (J. Martin, pers. comm.), *D. vexillum* was not.

The results of two subsequent Rapid Assessment surveys for *D. vexillum* conducted in DFO-Maritimes Region are presented herein: one in southwest NB in October of 2012, and one in a second "at-risk" area (Theriault and Herborg 2007) along the southwest coast of NS (Yarmouth to Shelburne) in September 2013.

2.0 Materials and Methods

2.1 Survey Locations and Stations

2.1.1 Southwest New Brunswick (swNB)

Eighteen sites were examined at low tide for the presence of *D. vexillum* in Passamaquoddy Bay and the Western Isles region of southwest NB, hereafter referred to as swNB, (Figure 1) on October 2-3, 2012, using several of the survey tools described below. These locations consisted of small to large harbours with public wharves, some with floating docks, and small coves without wharves (Table 1). Benthic dredging was conducted in another 21 locations (Figure 1, Table 2).

2.1.2 Southwest Nova Scotia (swNS)

Twenty-eight sites were examined for the presence of *D. vexillum* in the area between Yarmouth Bar and Shelburne (Figure 2, Table 3), hereafter referred to as swNS, between September 23 and 25, 2013, using several of the survey methods survey tools

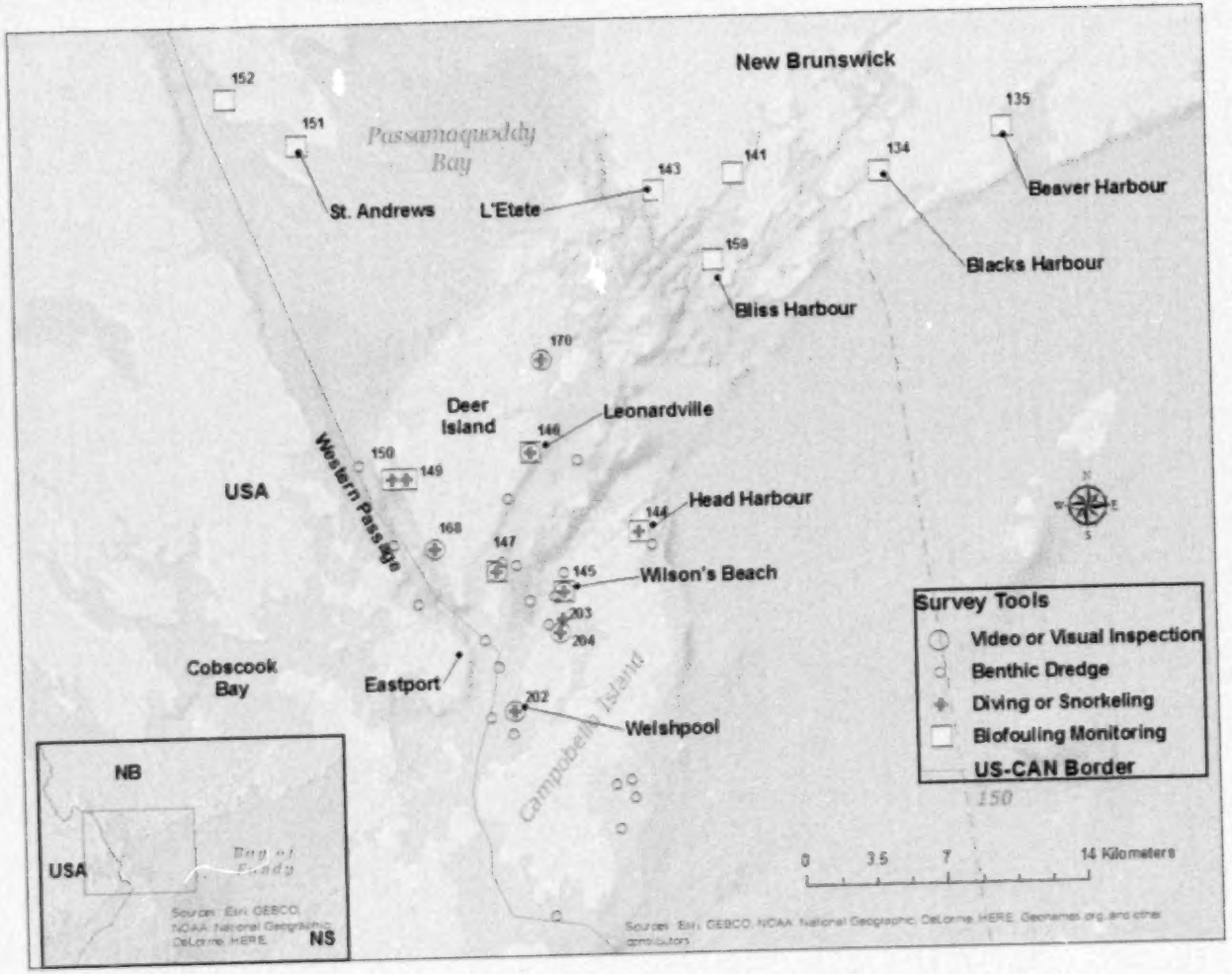

Figure 1. Sampling locations for the southwest New Brunswick Rapid Assessment Survey, October 2-3, 2012.

Table 1. Station list and survey tools used for the southwest New Brunswick Rapid Assessment surveys, October 2-3, 2012. CI = Campobello Island and DI = Deer Island. Survey tools used were: biofouling monitoring (BFM), environmental profiling (EP), scuba diving (SD), snorkeling (S), video camera (V), and visual inspection (VI).

Station	Stn ID	Station Description	Latitude	Longitude	Survey Tools Used
Fairhaven (DI)	149	Public wharf	44.9640	-67.0079	BFM, EP, SD, S
Fairhaven Marine Fish (DI)	150	Finfish Aquaculture Site	44.9640	-67.0143	BFM, SD
Leonardville (DI)	146	Public wharf	44.9716	-66.9527	BFM, EP, S, SD, V
Lord's Cove (DI)	170	Small Cove	45.0004	-66.9472	EP, S, SD, V
Cummings Cove (DI)	168	Small Cove	44.9425	-66.9958	EP, SD, V
Indian Island	147	Finfish Aquaculture Site	44.9261	-66.9667	BFM, EP, SD, V
Head Harbour (CI)	144	Public wharf	44.9466	-66.9055	BFM, EP, SD
Wilson's Beach (CI)	145	Public wharf	44.9283	-66.9390	BFM, EP, SD, VI
Curry Cove (CI)	203	Public wharf	44.9279	-66.9385	EP, S
Man O' War Head (CI)	204	Public wharf	44.9160	-66.9414	EP, S,V
Welshpool (CI)	202	Public wharf	44.8919	-66.9624	EP, SD, VI
St. Andrews Biological Station (SABS)	152	Private wharf; Government Research Station	45.0823	-67.0847	BFM
St. Andrews	151	Public wharf	45.0677	-67.0531	BFM
L'Etete	143	Public wharf, ferry landing	45.0513	-66.8955	BFM
Black's Harbour	134	Public wharf, ferry landing	45.0559	-66.7948	BFM
Bliss Harbour	159	Aquaculture site	45.0296	-66.8664	BFM
Back Bay	141	Aquaculture site	45.0561	-66.8638	BFM
Beaver Harbour	135	Public wharf	45.0688	-66.7398	BFM

Table 2. Locations of benthic (scallop drag) surveys for the southwest New Brunswick Rapid Assessment surveys in 2012. CI = Campobello Island, DI = Deer Island, INT = Canada-USA International Line, and ME = Maine, USA. Depth (m) gives depth at start and end of tows as a range.

Tow #	Tow Name	Tow Start Latitude	Tow Start Longitude	Tow End Latitude	Tow End Longitude	Depth (m)
1	Welshpool (CI)	44.89010	-66.96218	44.88295	-66.96529	24-21
2	Friars Rd (CI)	44.90271	-66.96951	44.90879	-66.96844	32-39
3	Eastport by INT Line (ME)	44.91252	-66.97105	44.91679	-66.97791	50-81
4	Malloch Beach (CI)	44.91712	-66.94412	44.92064	-66.94955	18-27
5	Windmill Point (CI)	44.92555	-66.94237	44.92852	-66.94514	27-28
6	Wilson's Beach (CI)	44.93112	-66.94228	44.93660	-66.93665	16-23
7	Head Harbour Island (CI)	44.94511	-66.89877	44.94933	-66.90829	19-14
8	Cummings/Johnsons Cove (DI)	44.93540	-67.00155	44.94133	-67.00607	45-59
9	Cummings Cove (DI)	44.94451	-67.00723	44.95329	-67.02052	70-72
10	Cummings Cove (DI)	44.96364	-67.02654	44.97363	-67.03238	70-76
11	Eastport to Lubec (ME)	44.89767	-66.97142	44.88493	-66.97490	33-24
12	Lower Duck Pond (CI)	44.82808	-66.95161	44.82893	-66.94043	9-9
13	Dinner Cove (CI)	44.85088	-66.91859	44.86000	-66.91575	18-16
14	Herring Cove North (CI)	44.86572	-66.92249	44.87183	-66.91514	11-16
15	Herring Cove (CI)	44.87159	-66.91223	44.86869	-66.90931	24-33
16	Herring Cove (CI)	44.86857	-66.90876	44.85963	-66.91047	35-30
17	Sandy Ledge (DI)	44.96586	-66.92257	44.96201	-66.93881	77-56
18	Hibernia Cove (DI)	44.96009	-66.95924	44.95150	-66.96585	34-30
19	Indian Head (DI)	44.94087	-66.96844	44.93608	-66.96388	24-16
20	Indian Head (DI)	44.93451	-66.95756	44.94033	-66.96346	39-30
21	Indian Island to Windmill Point (DI) (shipping lane)	44.92801	-66.95412	44.92393	-66.95428	68-80

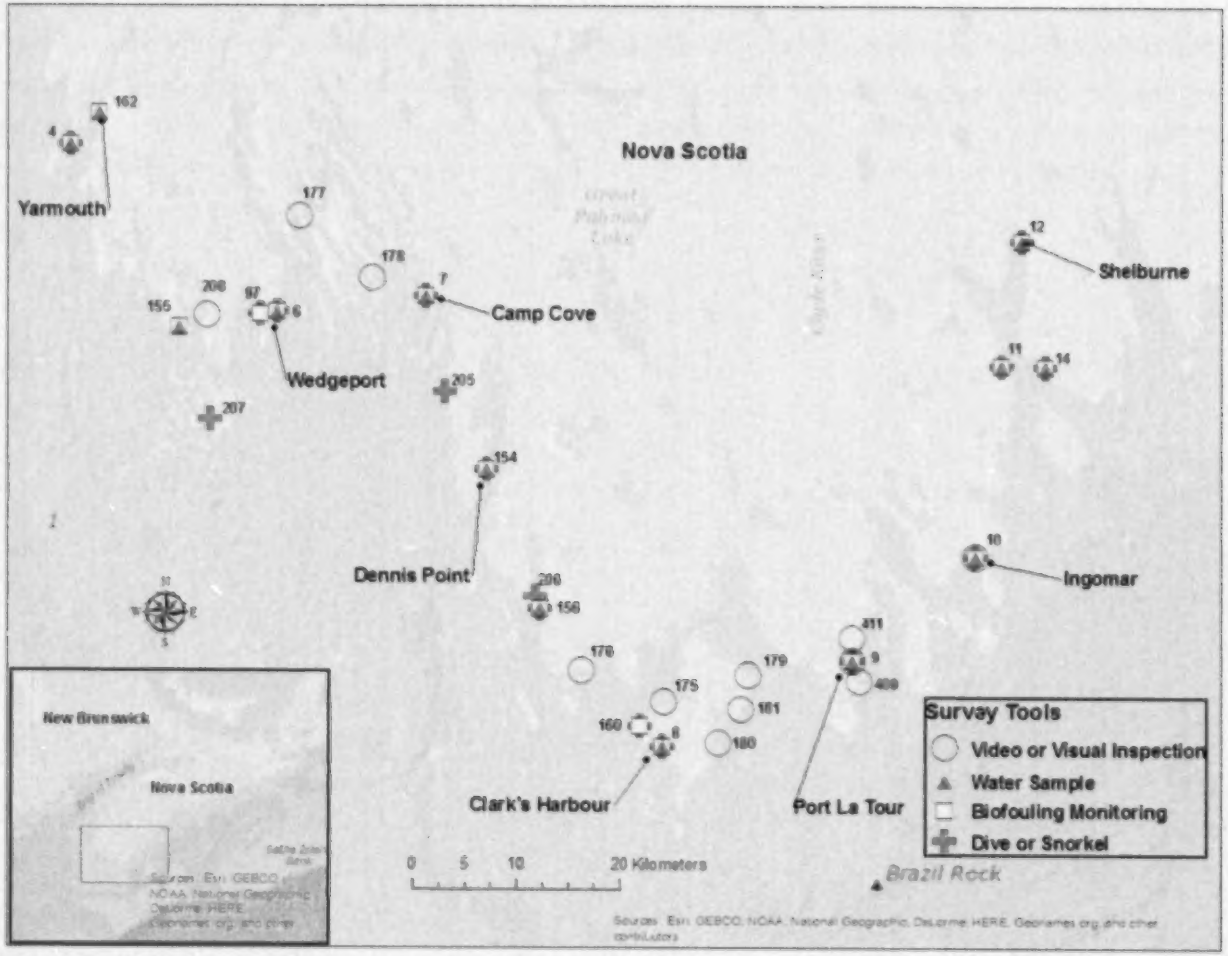

Figure 2. Location of the stations for the southwest Nova Scotia Rapid Assessment Surveys, September 23-25, 2013.

Table 3. Station list and survey tools used for the southwest Nova Scotia Rapid Assessment surveys, September 23-25, 2013. Survey tools used were: biofouling monitoring (BFM), environmental profiling (EP), scuba diving (SD), snorkeling (S), video camera (VC), visual inspection (VI), and water sampling(WS).

Station	Stn ID	Station Description	Latitude	Longitude	Survey Tools
Yarmouth Bar	4	Public wharf	43.81648	-66.14785	BFM, EP, SD, WS
Yarmouth Yacht Club	162	Private marina	43.83630	-66.12328	BFM, EP, WS,
Pinkney's Point	155	Public wharf	43.70423	-66.05459	BFM, EP, WS,
Little River Harbour	208	Public wharf	43.71170	-66.03080	EP, VI
Harris Island	207	Public wharf	43.64716	-66.02766	SD
Wedgeport Tuna Wharf	97	Public wharf	43.71204	-65.98434	BFM, SD
Wedgeport	6	Public wharf	43.71370	-65.96947	BFM, EP, SD, WS
Sluice Point	177	Public wharf	43.77280	-65.95030	VI
Morris Island	178	Public wharf	43.73470	-65.88730	EP, VI
Camp Cove	7	Public wharf	43.72360	-65.84057	BFM, EP, SD, S, WS
Abbott's Harbour	205	Public wharf	43.66408	-65.82349	SD
Dennis Point	154	Public wharf	43.61668	-65.78796	BFM, EP, SD, WS
Forbes' Point	206	Public wharf	43.53780	-65.74550	SD
Fall's Point	156	Public wharf	43.53010	-65.74178	BFM, EP, SD, WS
Shag Harbour	176	Public wharf	43.49227	-65.70524	VI
Newellton	175	Public wharf	43.47260	-65.63387	VI
West Head	160	Public wharf	43.45813	-65.65458	BFM, EP, SD
Clark's Harbour	8	Public wharf	43.44470	-65.63510	BFM, EP, SD, WS
South Side	180	Public wharf	43.44733	-65.58602	VI
Stoney Island	181	Public wharf	43.46804	-65.56720	VI
Cripple Creek	179	Public wharf	43.48892	-65.56040	VI
Smithsville	409	Public wharf	43.48567	-65.46350	VI
Port la Tour	9	Public wharf	43.49848	-65.46988	BFM. EP, SD, V, WS
Upper Port La Tour	411	Public wharf	43.56273	-65.36245	VI
Ingomar	10	Public wharf	43.56273	-65.36245	BFM, EP, SD, V, WS
Gunning Cove	11	Public wharf	43.68073	-65.33973	BFM, EP, SD, S, WS
Shelburne	12	Private marina	43.75780	-65.32200	BFM, EP, SD, S, WS
Lower Sandy Point	14	Public wharf	43.68013	-65.30135	BFM, EP, SD, S, WS

described below. These locations consisted of small to large harbours with public wharves, some with floating docks, and recreational marinas.

2.2 Scuba Dive and Snorkeling Surveys

In swNB, scuba diving teams used the Fisheries and Oceans vessel *Viola B. Davidson* as a diving platform. Divers worked in pairs to search the bottom under each wharf and about 1m beyond each side and any submerged structures at low tide (+/- 2 hours) at 8 locations (Table 1). Snorkeling surveys were conducted at 5 locations, using the New Brunswick Department of Agriculture, Aquaculture and Fisheries (NBDAAF) vessel *Colonel Barry* as a platform. Pairs of snorkelers examined the undersurfaces of floating docks, submerged structures, floats and buoys, and wharf pilings down to about 1 m swimming along both sides of each floating dock at each station.

In swNS, either a 24' steel hulled ABCO (ABCO Industries, Lunenburg, NS) vessel or floating docks were used as platforms to examine a total of 15 stations. Diving times were not restricted to low-tide, as was the case in swNB. Four snorkeling surveys as described above were conducted from shore or floating docks.

Divers and snorkelers were equipped with GoPro HERO (GoPro Inc., San Mateo, CA, USA) head-mounted, underwater cameras for *in situ* detection of AIS. They also carried wrist-mounted pictures for visual identification of *D. vexillum*, 3 other species of invasive tunicates, namely: *Ciona intestinalis* (Linnaeus 1776), *Botryllus schlosseri* (Pallas 1766) and *Botrylloides violaceus* (Oka 1927) known to be present in both regions (Martin et al. 2011; Sephton et al. 2011) and potential new invaders (Locke 2009), namely: *Styela clava* (Herdmann 1881), *Diplosoma listerianum* (Milne-Edwards 1841), and *Ascidiella aspersa* (Muller 1776) (Figure 3). Didemnid-like specimens were collected in mesh bags, returned to the surface, and held on ice until day's end for examination. Didemnid-like tissue samples were preserved in 10% seawater-formalin buffered with sodium borate to preserve calcium carbonate spicules (Martin et al. 2010), or in 95% ethanol for subsequent DNA tissue analysis.

2.3 Video Surveys and Visual Inspections

Video camera surveys were conducted using Micro Video ™ colour underwater inspection cameras, attached to a metal pole up to 6 m in length. Cyberlink and Microsoft Windows Movie Maker software were used to record camera images. Video clips were obtained as camera operators systematically panned both sides and the end of a wharf or floating dock, moving up and down at wharf pilings (Figure 4A). A computer operator viewed the images in real time, recording Didemnid-like individuals as they were observed. In some cases, a diver or snorkeler could be directed to the specific location to obtain a sample for examination and preservation as described above.

Figure 3. Scuba divers examining a floating dock, and recording AIS present.

Visual inspections were conducted using an underwater viewing cone (bathyscope) (Figure 4B), to examine submerged structures at depths of less than 0.25 m. Selected sections of floating docks (~5 m in length) were examined, and accessible submerged ropes, chains, floats or were inspected. Didemnid-like specimens were photographed and sampled, and then examined and preserved as described above.

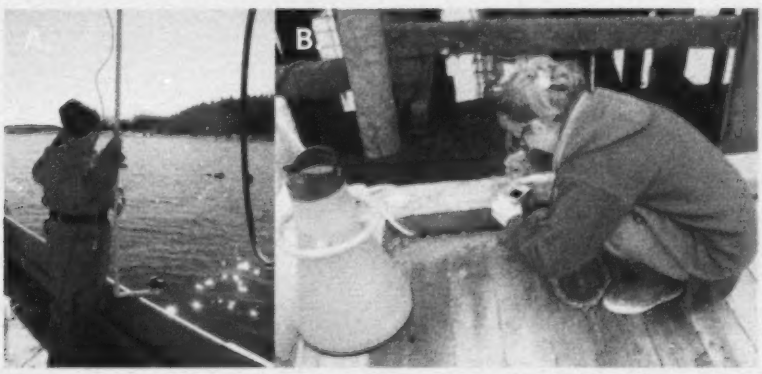

Figure 4. Video and visual inspection tools used during Rapid Assessments. A: Pole-mounted video camera, and B: Underwater viewing cone.

2.4 Benthic Dredge (Scallop Tow) Surveys – swNB Only

Benthic dredging was conducted at 21 locations (Table 3) on 2 and 3 October, 2012 aboard the *Fundy Spray* using a small scallop dredge (1 X 1 X 0.5 m Grand Manan "Miracle" gear). Samples were obtained by towing the dredge along the bottom (~10-80 m depth) for 5-8 min at low speed (~1 – 3.5 kn). Dredge contents were hand sorted on board, and didemnid-like tissues were examined and preserved as described above.

2.5 Biofouling Monitoring (BFM) Surveys

Many stations included in the Rapid Assessment surveys were DFO AIS BFM stations, where monitoring collectors had been deployed earlier in the year and were examined *in situ* to gather information on the presence (P) or absence (ND) of *D. vexillum*, and other non-indigenous tunicates: *C. intestinalis*, *B. schlosseri*, *B. violaceus*, *S. clava*, *D. listerianum* and *A. aspersa*.

In swNB, saucer-type collectors (Figure 5A) described by Martin et al. (2011) were used, while plate-type collectors (Figure 5B) were used in swNS (Sephton et al. 2014). Two collectors deployed in May and two collectors deployed in August (Full and Second Set, respectively, see Sephton et al. 2014) were examined during each survey.

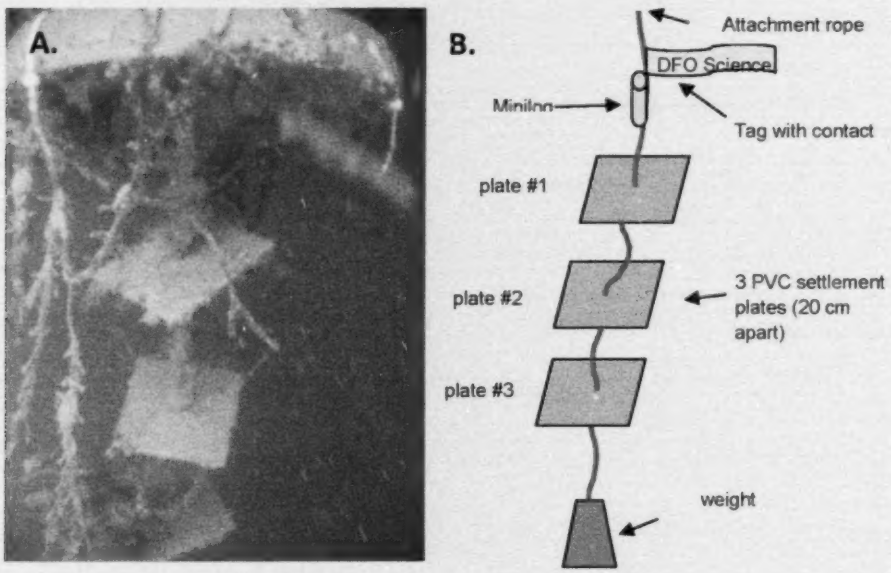

Figure 5. A. Saucer-type biofouling monitoring collector *in situ* and B. Diagram of plate-type monitoring collector.

Collectors were placed so that either the saucer, or the top plate (plate #1) was 1 m below the water surface. Nearby structures were also examined for the presence of tunicates using an underwater viewing cone (bathyscope).

2.6 Water Sampling and genetic screening for tunicate species– swNS only

Water samples were collected to detect the presence (P) of *D. vexillum* larvae at 13 stations (Figure 2, Table 3) in swNS only. Samples were obtained in triplicate at each station; one from the end of the dock, one off the left and one off the right side if one dock only was present, or from the end of 3 separate docks if multiple docks were present at the station. A submersible pump, calibrated on site to determine the pumping time required, was used to collect 150L of water from a depth of 1m. Water was pumped through a 63-µm mesh sieve to collect any tunicate larvae present, and sieve contents were rinsed into clean 15 or 50 mL Falcon tubes with filtered seawater, held on ice and frozen within 12h. The frozen samples were delivered to the Aquaculture Genomics Laboratory, Dalhousie University, Truro, NS for PCR-based (polymerase chain reaction) DNA analysis for the detection of *D. vexillum* (Vercaemer et al. 2015). PCR-based assays were also used to screen for the presence of *D. listerianum* (Willis et al. 2011), *B. schlosseri, C. intestinalis, S. clava*, and *B. violaceus* (Stewart-Clark et al. 2009).

2.7 Environmental Profiling

Depth profiles (surface to bottom) of water column physical variables were made using either a YSI (Yellow Springs Instruments Incorporated, Yellow Springs, Ohio, USA) Castaway Unit (measuring temperature in °C and salinity in psu; swNB survey only), or a YSI 6600 Sonde (measuring temperature in °C , salinity in psu, dissolved oxygen in % saturation and mg l^{-1}, pH, and chlorophyll a in µg l^{-1}). Probe calibration was conducted as per the manufacturer's instructions prior to each survey for all parameters. The Castaway Unit is designed to free fall through the water column at a speed of 1 m sec^{-1} measuring parameters every 0.3 m, while the YSI 6600 Sonde is hand lowered and measures parameters every 10 s. All raw data collected were downloaded using YSI Ecowatch software for presentation in tabular form (Appendix 1).

3.0 Results and Discussion

3.1 swNB, 2012

3.1.1 Non-detection of *Didemnum vexillum*

Didemnum vexillum was not detected at any station by any of the survey and detection techniques used in 2012, nor during previous annual rapid assessments conducted in 2009 (Martin et al. 2010), 2010 and 2011 (J. Martin, pers. comm.). Water column temperature and salinity values measured during the survey (Appendix 1) were within

the range of environmental tolerances of the species (Daniel and Therriault 2007). Despite its presence nearby in Eastport, ME, within 2 km of stations surveyed on Campobello and Deer Island, *D. vexillum* was not detected in these areas, nor has it been detected further afield in the St. Andrews Area (upper reaches of Passamaquoddy Bay), or along the coast of swNB at biofouling monitoring stations at Bliss Harbour, Black's Harbour and Beaver Harbour (Sephton et al. 2015; unpubl. data).

Several potential mechanisms of spread into this region from Eastport, ME have been identified. Chang et al. (2005) used tidal-circulation and particle transport models to study water circulation (currents and tidal movements) in Cobscook Bay, ME and swNB to determine the potential for water exchange of the ISA (infectious salmon anaemia) virus among salmon farms in the region. They found that the tidal excursion areas from salmon farms on Moose Island, where Eastport is located, extended eastward and along the western shore of Campobello Island (CI), and north to southern Deer Island (DI), and that there was also considerable exchange between the Deer Island and Campobello sites. It is possible then, that floating *D. vexillum* fragments dislodged from the wharf at Eastport may reach Deer and Campobello Islands via current or tidal movements. Transport of *D. vexilllum* attached to vessel hulls (recreational or commercial) or salmon aquaculture gear moving from site to site within the Western Isles and into Passamaquoddy Bay, L'Etete and Bliss Harbours is also possible (Martin et al. 2011). Dislodgement of *D. vexillum* attached to bottom substrate by scallop dragging in Western Passage, around Campobello and Deer Islands or off Grand Manan Island in Scallop Production Area 6 (SPA6) may also facilitate its spread, although it was not detected during a recent survey in this area (Vercaemer et al. 2015).

3.1.2 Presence of Didemnids and other non-indigenous tunicate species

The presence of *D. albidum* (Verrill 1871), the northern white-crust tunicate, a common, indigenous New England (Lambert 2009) and Bay of Fundy (Gosner 1971) species, was noted in 6 of 21 dredge samples (Table 4) taken during the survey, and it was also observed at Leonardville (DI) and Head Harbour (CI) during diving surveys. These were visual detections only, and no samples were taken for taxonomic identification or molecular analysis, so these results were not definitive. However, *D. albidum* is quite different from *D. vexillum* in colour (white as opposed to creamy yellow or tan, Daniel and Therriault 2007; Lambert 2009). Colonies of *D. albidum* grow in flat, thin, encrusting sheets never more than 3 mm thick, while *D. vexillum* colonies overgrow surfaces and one another, often forming extensive, thick mats or long, flat, leaf or frond-growths (Daniel and Therriault 2007) (Figure 6) .

Table 4. Benthic dredge samples where *D. albidum* was present (P).

Tow #	Tow Name
2	Friars Rd (Campbobello Island)
4	Malloch Beach (Campobello Island)
6	Wilson's Beach (Campobello Island)
8	Cummings/Johnsons Cove (Deer Island)
9	Cummings Cove (Deer Island)
15	Herring Cove (Campobello Island)

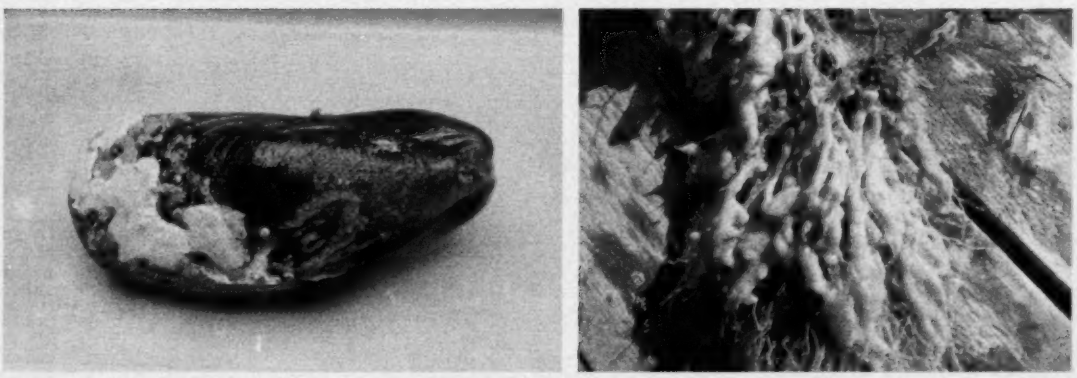

Figure 6: Colonies of *D. albidum* growing on a blue mussel, *Mytilus edulis* (left), and *D. vexillum* from Eastport, ME, showing frond-like growths (right).

One small (<4 cm diameter), creamy-tan Didemnid colony found on a rock between two pilings at the public wharf at Welshpool (CI) in about 3.5 m of water was collected during the survey that warranted identification. It was held on ice, and examined at SABS later that day. A thin (< 1 mm) slice of tissue was soaked in bleach (5% sodium hypochlorite) for ~30 min, rinsed with water and examined under a compound microscope at 10X, to examine its spicules, a morphological character often used in taxonomic identification of Didemnid species (Lambert 2009). Unfortunately, preliminary taxonomic identification of the sample failed, as spicule diameter (Figure 7) was not recorded, and comparison with published values for various Didemnid species (Daniel and Therriault 2007) could not be made. Stephaniak et al. (2012) noted, however, that there are considerable morphological similarities in taxonomic characters among species in the genus *Didemnum*, and the use of molecular markers is required to identify species when taxonomic characters are questionable.

The Welshpool didemnid tissue was tested using molecular analysis of the 18S gene (S. Stewart-Clark, pers. comm.), which indicated that the sample was not *D. vexillum*. Unfortunately, the assay could not determine if the specimen was *D. albidum*, or another Didemnid species. In addition to *D. albidum*, *D candidum* (Savigny 1816) is present in this area (Daniel and Therrriault 2007; Gosner 1971), and it is possible that another species, *D. lutarium* (Van Name 1910), which was widespread and abundant in New England waters as far north as Maine up until the early 1900's (Lambert 2009) may also be present. Future monitoring and survey work in swNB should focus on the collection of didemnid-like tissues for taxonomic examination, comparison with archived samples, and the development of species specific molecular assays which will clarify the native didemnids present in this area.

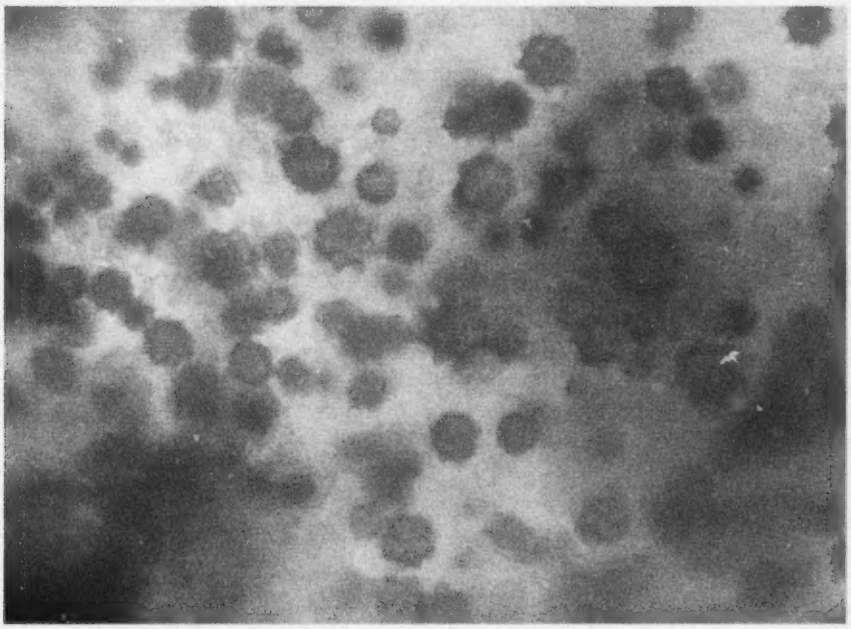

Figure 7. *Didemnum* sp. spicules from a small colony collected at Welshpool, NB, October 3, 2012, viewed under a compound microscope at 10X. Spicule diameter was not recorded.

Ciona intestinalis, *B. schlosseri* and *B. violaceus* were detected at several stations during the survey (Appendix 2).

3.2 swNS, 2013

3.2.1 Non-detection of *Didemnum vexillum*

Didemnum vexilum was not detected at any station surveyed in swNS during the 2013 survey. Water column temperature and salinity values measured during the survey (Appendix 3) were within its range of environmental tolerances (Daniel and Therriault 2007). It was not present on biofouling monitoring collectors, although other species of non-indigenous solitary and colonial tunicates were (Appendix 4), nor was it observed during snorkelling and diving surveys. Larvae of *D. vexillum* were not detected by molecular analysis of water samples collected at 13 stations, although those of *B. schlosseri* and *B. violaceus* were (Appendix 5).

Many sites surveyed in 2013 were sentinel biofouling monitoring sites that have been monitored annually between 2006 and 2014, and while several species of non-indigenous tunicates have been established on the southwest coast for many years (Carver et al. 2003; Carver et al. 2006), *D. vexillum* has never been detected. Lacoursiere-Roussel et al. (2012a) identified the ports of Yarmouth and Shelburne as potential sites of new introduction of *B. schlosseri*, and potentially other colonial tunicates, based on the number of annual international ship arrivals (178 and 56, in 2009) to these ports. Moore et al. (2014) also identified this region, along with the south shore of Nova Scotia extending to Halifax, and the Bay of Fundy as vulnerable to new invasions of *D. vexillum* from eastern US waters mediated through shipping, recreational boating and northward range-expansions. This area is subject to prevailing oceanographic currents from US waters, so *D. vexillum* could be introduced as floating fragments dislodged during scallop fishing activities on the US side of Georges Bank. There are also many home ports to the offshore scallop fleet in this area (SFA 29), so it is possible that *D. vexillum* might be introduced as fragments trapped in fishing gear.

4.0 Concluding Remarks

Despite extensive recent survey efforts in the Western Isles Region of the BoF (swNB), and between Yarmouth and Shelburne (swNS), *D. vexillum* has not been detected in nearshore coastal waters in these areas. BFM conducted in both of these regions since 2006 has also failed to detect this species. The collectors used are effective in the detection of didemnids, as small colonies of *D. albidum* were detected on plates deployed in swNB between 2006 and 2009 (Martin et al. 2011). Both regions have been identified as "high risk" for the introduction and establishment of this species

based on matching environmental conditions in established and potential habitats (Therriault and Herborg 2007), and on the existence of multiple vectors of introduction and secondary spread (Lacoursiere-Roussel et al. 2012b) .

In 2013, *D. vexillum* was confirmed in the upper reaches of the BoF, in the Minas Basin off Parrsoboro, NS (Moore et al. 2014). Extensive survey work was carried out in 2014 to delineate and confirm its presence in other regions of the BoF, as well as several offshore banks frequented by scallop vessels from the Atlantic Canadian offshore fleet (Vercaemer et al. 2015). It was concentrated in the upper reaches of the BoF, with two additional locations: one off Digby Gut (mid-BoF), and one off Yarmouth (outer BoF), and with no occurrences in the western Fundy Isles Region. This introduction may have been facilitated by vessel (barge) traffic from northeastern US ports with secondary spread in the upper BoF facilitated by rafting or re-attachment of floating fragments (Vercaemer et al. 2015). Vessels of the Inshore Scallop fleet move into this area, and throughout the BoF, and the predominant pattern of circulation is counterclockwise from the NS side of the Bay along the NB shore to swNB (Aretxabaleta et al. 2008) so there is potential for spread from the upper BoF throughout the Bay and into swNB.

We cannot confirm the absence of *D. vexillum* off the southwest and south shores of NS at present, as extensive surveys of the type described by Vercaemer et al. (2015) have not been conducted there. Scallop fishermen in this area (SFA 29) have been provided with identification and collection materials, so the species may be recognized and reported if it is present, although no Didemnid-like tissues were reported there during a scallop survey in September 2014 (A. Glass, pers. comm). The extensive areas of gravel/sand habitat found in this region should be surveyed, and new benthic, artificial substrate biofouling monitoring collectors successfully tested in the Minas Basin in 2014 will be deployed in this region in 2015.

The potential ecological impact of *D. vexillum* on bottom substrata, benthic invertebrates (Bullard et al. 2007; Lengyel et al. 2009; Valentine et al. 2007b) and fisheries (Valentine et al. 2007a; Morris et al. 2009) have been w ell documented. Atlantic Canadian waters in general are at "high-risk" of ecological consequences if this tunicate is introduced and establishes (Therriault and Herborg 2007). Given its presence in two regions of the Bay of Fundy, it is important that early detection efforts and monitoring of spread throughout the Bay continue, especially in ports and harbours where shipping, scallop fishing or recreational boating are active. In Nova Scotia, early detection and monitoring should focus on ports and marinas along the southwest and southern coast with heavy US vessel (recreational and commercial) traffic from areas where *D. vexillum* is present, given that recent introductions of three non-indigenous tunicate species have occurred there (Lunenburg), and also in Halifax harbor (Moore et al. 2014). Analysis of

vessel traffic between infected sites in the Bay of Fundy, particularly the Minas Basin, and other regions in Atlantic Canada, may identify additional ports that are at risk of introduction of *D. vexillum*, and inform strategies to contain, mitigate and manage the impact of this species (Vercaemer et al. 2015).

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Appendix 1: Water column physical variables measured during the swNB Rapid Assessment Survey, 2-3 October 2012. Stn = Station, m = meters, T = temperature, SpCond = specific conductivity, Cond =Conductivity, Chla = chlorophyll a, NA = Not applicable, NM = Not measured.

Station and Instrument	Stn #	Date	Depth m	Temp °C	SpCond mS/cm	Cond mS/cm	Salinity psu	pH	Chla µg/L	Oxygen %	Oxygen mg/L
Cummings Cove	168	2-Oct	0.62	13.68	48.78	38.24	31.84	7.94	5.32	93.30	7.95
YSI Sonde 6600			1.19	13.68	48.79	38.24	31.84	7.94	3.76	90.60	7.72
			3.48	13.68	48.83	38.27	31.87	7.94	3.82	90.60	7.72
			4.86	13.68	48.86	38.30	31.90	7.94	3.98	90.30	7.69
			5.44	13.68	48.89	38.32	31.91	7.93	3.82	89.90	7.66
			2.41	13.68	48.89	38.32	31.91	7.93	3.71	89.80	7.65
FairHaven	149	2-Oct	1.87	13.61	48.95	38.30	31.96	7.97	5.21	89.60	7.64
YSI Sonde 6600			2.13	13.61	48.96	38.31	31.96	7.97	4.95	89.50	7.63
			4.40	13.61	49.04	38.37	32.02	7.96	5.21	89.20	7.61
			4.99	13.60	49.18	38.47	32.13	7.95	6.45	88.30	7.52
			4.00	13.60	49.16	38.46	32.11	7.95	6.77	87.10	7.42
			2.27	13.61	49.01	38.34	32.00	7.96	5.75	86.90	7.41
			0.95	13.61	48.95	38.30	31.96	7.97	5.43	87.60	7.47
FairHaven	149	2-Oct	0.15	13.59	48.22	37.22	30.97				
CastAway			0.45	13.63	48.23	37.26	30.98				
			0.75	13.63	48.01	37.09	30.82				
			1.05	13.64	48.10	37.17	30.89				
			1.35	13.65	47.98	37.09	30.80				
			1.65	13.65	47.68	36.86	30.58				
			1.94	13.67	47.38	36.64	30.37				
			2.24	13.67	47.21	36.52	30.25				
			2.54	13.67	47.32	36.60	30.33				
			2.84	13.67	47.47	36.72	30.44				
			3.14	13.68	47.39	36.66	30.38				
			3.44	13.66	47.54	36.76	30.48				
			3.79	13.69	46.98	36.35	30.09				
Head Harbour	144	2-Oct	0.95	13.69	49.43	38.76	32.31	7.94	5.35	90.20	7.66
YSI Sonde 6600			1.16	13.69	49.43	38.75	32.31	7.94	5.35	90.20	7.66
			1.93	13.67	49.49	38.78	32.35	7.94	5.35	90.20	7.66
			3.52	13.65	49.58	38.83	32.42	7.94	7.77	89.20	7.57
			5.86	13.60	49.78	38.94	32.56	7.95	7.99	89.00	7.56
			7.52	13.59	49.83	38.97	32.60	7.95	8.28	88.90	7.55
			8.83	13.59	49.84	38.97	32.60	7.95	8.28	89.00	7.56

Appendix 1, continued.

Station and Instrument	Stn #	Date	Depth m	Temp °C	SpCond mS/cm	Cond mS/cm	Salinity psu	pH	Chla µg/L	Oxygen %	Oxygen mg/L
Leonardville	146	3-Oct	1.09	13.59	49.84	38.98	32.61	7.93	2.27	93.10	7.91
YSI Sonde 6600			2.19	13.56	49.86	38.97	32.62	7.93	2.27	93.10	7.91
			3.92	13.55	49.92	39.00	32.66	7.93	2.27	93.10	7.91
			5.36	13.53	49.95	39.01	32.68	7.93	3.08	88.60	7.54
			5.20	13.53	49.96	39.02	32.69	7.93	3.59	88.10	7.49
			3.51	13.54	49.93	39.00	32.67	7.93	3.59	87.50	7.44
			1.43	13.54	49.88	38.97	32.63	7.93	3.23	87.50	7.44
Leonardville	146	2-Oct	0.15	13.71	48.75	37.75	31.36				
CastAway			0.45	13.71	48.80	37.78	31.39				
			0.75	13.71	48.81	37.79	31.40				
			1.05	13.71	48.82	37.79	31.40				
			1.35	13.70	48.87	37.83	31.44				
			1.64	13.71	48.93	37.89	31.49				
			1.94	13.71	49.02	37.95	31.55				
			2.24	13.71	49.05	37.98	31.57				
			2.54	13.72	49.10	38.02	31.60				
			2.84	13.73	49.14	38.07	31.63				
			3.20	13.75	49.13	38.07	31.63				
Lord's Cove	170	3-Oct	1.10	13.84	49.59	39.02	32.43	7.93	NM	90.50	7.66
YSI Sonde 6600			1.22	13.83	49.60	39.02	32.44	7.94	1.98	90.60	7.66
			2.00	13.82	49.62	39.03	32.45	7.94	2.64	90.10	7.62
			2.94	13.81	49.66	39.05	32.48	7.93	2.79	89.70	7.59
			3.69	13.81	49.67	39.06	32.49	7.93	2.86	89.10	7.54

Appendix 1, continued.

Station and Instrument	Stn #	Date	Depth m	Temp °C	SpCond mS/cm	Cond mS/cm	Salinity psu	pH	Chla µg/L	Oxygen %	Oxygen mg/L
Lord's Cove	170	2-Oct	0.15	14.19	48.88	38.31	31.49				
YSI CastAway			0.45	14.07	49.21	38.46	31.71				
			0.75	14.01	49.67	38.75	32.04				
			1.05	13.98	50.16	39.11	32.39				
			1.34	13.96	50.40	39.28	32.56				
			1.64	13.96	50.34	39.22	32.52				
			1.94	13.94	50.28	39.16	32.47				
			2.25	13.95	50.23	39.13	32.44				
			2.54	13.93	50.26	39.14	32.46				
			2.84	13.94	50.28	39.16	32.47				
			3.14	13.92	50.28	39.14	32.47				
			3.44	13.92	50.31	39.16	32.49				
			3.73	13.92	50.31	39.17	32.49				
			4.03	13.92	50.33	39.17	32.51				
			4.33	13.93	50.35	39.20	32.52				
			4.63	13.92	50.36	39.20	32.53				
			4.93	13.92	50.37	39.21	32.53				
			5.23	13.91	50.38	39.21	32.54				
			5.53	13.92	50.39	39.22	32.55				
			5.83	13.93	50.32	39.18	32.50				
			6.12	13.94	50.31	39.19	32.49				
			6.57	13.95	50.11	39.04	32.35				
Welshpool	202	3-Oct	0.99	13.81	49.22	38.70	32.16	7.93	7.84	93.10	7.90
YSI Sonde 6600			0.99	13.80	49.21	38.68	32.15	7.93	7.84	93.20	7.90
			1.19	13.79	49.15	38.63	32.11	7.93	7.84	93.20	7.91
			2.83	13.76	49.18	38.62	32.13	7.93	7.04	90.10	7.65
			4.22	13.75	49.21	38.63	32.15	7.93	7.04	89.00	7.63
			5.70	13.75	49.21	38.63	32.15	7.93	6.89	89.60	7.61
			6.18	13.75	49.21	38.63	32.15	7.92	6.60	89.40	7.59
Wilsons Beach	145	3-Oct	1.12	13.70	49.46	38.78	32.33	7.91	6.60	91.00	7.73
YSI Sonde 6600			2.35	13.67	49.45	38.75	32.32	7.91	6.60	91.10	7.74
			4.61	13.62	49.51	38.75	32.36	7.92	6.60	91.10	7.75
			6.02	13.58	49.58	38.76	32.41	7.92	11.73	88.60	7.53
			6.06	13.57	49.59	38.76	32.42	7.92	11.07	88.40	7.52

Appendix 1, continued.

Station and Instrument	Stn #	Date	Depth m	Temp °C	SpCond mS/cm	Cond mS/cm	Salinity psu	pH	Chla µg/L	Oxygen %	Oxyge mg/L
Man O' War Head	204	3-Oct	0.15	13.61	50.09	38.68	32.30				
YSI CastAway			0.45	13.64	50.09	38.71	32.31				
			0.75	13.65	50.17	38.78	32.37				
			1.05	13.66	50.20	38.81	32.39				
			1.34	13.69	50.33	38.94	32.49				
			1.64	13.70	50.40	39.02	32.54				
			1.94	13.73	50.47	39.09	32.59				
			2.24	13.71	50.46	39.07	32.58				
			2.54	13.67	50.45	39.01	32.57				
			2.84	13.64	50.49	39.02	32.60				
			3.14	13.64	50.52	39.04	32.62				
			3.44	13.64	50.53	39.05	32.62				
			3.73	13.65	50.54	39.06	32.63				
			4.03	13.64	50.60	39.10	32.67				
			4.32	13.69	50.62	39.17	32.70				
Curry Cove	203	3-Oct	0.15	13.69	50.55	39.12	32.65				
YSI CastAway			0.45	13.69	50.54	39.11	32.64				
			0.75	13.69	50.55	39.11	32.64				
			1.05	13.69	50.54	39.11	32.64				
			1.34	13.69	50.54	39.11	32.64				
			1.64	13.69	50.55	39.12	32.64				
			1.94	13.69	50.55	39.12	32.64				
			2.24	13.69	50.55	39.11	32.64				
			2.54	13.67	50.59	39.13	32.67				
			2.84	13.65	50.62	39.13	32.69				
			3.14	13.63	50.64	39.12	32.70				
			3.43	13.61	50.67	39.13	32.72				
			3.73	13.56	50.76	39.15	32.78				
			3.89	13.55	50.78	39.15	32.80				

Appendix 2: Presence of *Didemnum vexillum*, and other non-indigenous tunicate species on biofouling monitoring collectors examined *in situ* in swNB, 2-3 October 2012. Stn # = station number, ***D. v.*** = *Didemnum vexillum*, ***C. i.*** = *Ciona intestinalis*, ***B. s.*** = *Botryllus schlosseri*, ***B. v.*** = *Botrylloides violaceus*, ***S. c.*** = *Styela clava*, ***D. l.*** = *Diplosoma listerianum* and ***A. a.*** = *Ascidiella aspersa*. ND = Not detected, P = Present, DI = Deer Island and CI = Campobello Island.

Station	**Stn #**	***D. v.***	***C. i.***	***B. s.***	***B. v.***	***S. c.***	***D. l.***	***A. a.***
Fairhaven (DI)	149	ND	P	P	ND	ND	ND	ND
Fairhaven MF (DI)	150	ND	P	P	ND	ND	ND	ND
Leonardville (DI)	146	ND	P	P	ND	ND	ND	ND
Indian Island	147	ND	P	ND	ND	ND	ND	ND
Head Harbour (CI)	144	ND	P	P	P	ND	ND	ND
Wilsons Beach (CI)	145	ND	P	P	P	ND	ND	ND
SABS	152	ND	P	P	ND	ND	ND	ND
St. Andrews	151	ND	P	P	ND	ND	ND	ND
L'Etete	143	ND	P	ND	ND	ND	ND	ND
Black's Harbour	134	ND	P	ND	ND	ND	ND	ND
Bliss Harbour	159	ND	P	ND	ND	ND	ND	ND
Back Bay	141	ND	ND	ND	ND	ND	ND	ND
Beaver Harbour	135	ND	P	P	P	ND	ND	ND
Present/Total Stations		**0 /14**	**13/14**	**8/14**	**3/14**	**0 /14**	**0/14**	**0 /14**

Appendix 3: Water column physical variables measured during the swNS Rapid Assessment Survey, 23-25 September 2013. Stn = Station, m = meters, T = temperature, SpCond = specific conductivity, Cond = Conductivity, Chla = chlorophyll a, NA = Not applicable, NM = Not measured.

Station and Instrument	Stn #	Date	Depth m	Temp °C	SpCond mS/cm	Cond mS/cm	Salinity psu	pH	Chla µg/L	Oxygen %	Oxygen mg/L
Pinkney's Point	155	23-Sep	0.91	13.21	43.98	34.07	28.36	7.81	3.89	98.70	8.68
			1.19	13.19	43.89	33.99	28.30	7.80	3.89	98.70	8.69
			3.55	13.10	43.87	33.90	28.28	7.79	8.14	97.90	8.63
			2.98	13.05	43.89	33.87	28.29	7.78	14.95	97.50	8.60
			2.08	13.06	43.85	33.85	28.26	7.78	10.63	97.50	8.60
			1.56	13.13	43.83	33.89	28.25	7.79	8.58	97.90	8.63
			1.57	13.12	43.87	33.92	28.28	7.79	7.70	98.20	8.65
			1.57	13.14	43.87	33.94	28.28	7.79	7.04	98.20	8.65
			1.57	13.15	43.88	33.95	28.29	7.79	6.75	98.30	8.65
			1.57	13.15	43.89	33.96	28.29	7.79	6.97	98.10	8.64
			1.57	13.15	43.89	33.96	28.30	7.79	7.04	98.10	8.64
			1.57	13.16	43.90	33.97	28.30	7.79	7.19	98.20	8.65
Yarmouth Bar	4	23-Sep	1.26	12.41	40.65	30.88	25.97	8.00	3.96	98.70	8.95
			1.69	12.39	42.68	32.40	27.41	8.02	4.77	98.70	8.88
			4.24	12.24	42.96	32.49	27.60	8.03	4.40	98.70	8.90
			5.34	12.05	43.25	32.55	27.80	8.03	5.21	98.60	8.91
			3.59	12.05	43.40	32.66	27.91	8.03	6.23	98.70	8.91
			1.36	12.23	43.28	32.72	27.83	8.03	5.13	99.30	8.94
			1.44	12.38	43.35	32.90	27.89	8.03	4.77	99.60	8.93
			1.35	12.40	43.42	32.97	27.94	8.03	4.25	99.70	8.94
			1.35	12.41	43.49	33.03	27.99	8.03	3.96	99.70	8.93
			1.35	12.39	43.57	33.08	28.04	8.03	4.11	99.70	8.94
			1.35	12.38	43.63	33.12	28.09	8.03	4.18	99.70	8.93
			1.34	12.38	43.68	33.16	28.12	8.03	3.89	99.50	8.92

Appendix 3, continued.

Station and Instrument	Stn #	Date	Depth m	Temp °C	SpCond mS/cm	Cond mS/cm	Salinity ppt	pH	Chla µg/L	Oxygen %	Oxygen mg/L
Yarmouth Yacht Club	162	23-Sep	0.52	14.43	42.59	33.99	27.40	7.85	5.87	99.40	8.58
			1.35	14.28	42.84	34.07	27.58	7.86	5.87	99.60	8.61
			1.81	13.32	43.98	34.16	28.37	7.84	5.87	100.90	8.85
			2.79	12.98	44.19	34.05	28.50	7.84	5.87	101.40	8.94
			3.19	12.83	44.28	33.99	28.56	7.83	5.06	97.20	8.60
			6.43	12.73	44.30	33.91	28.57	7.83	4.62	97.40	8.64
			7.37	12.68	44.33	33.89	28.59	7.83	7.11	97.50	8.65
			5.63	12.68	44.29	33.86	28.56	7.82	5.94	97.40	8.65
			3.35	12.70	44.19	33.81	28.50	7.82	5.28	97.80	8.68
			1.19	12.95	44.11	33.96	28.45	7.83	4.69	98.60	8.72
			1.66	13.46	43.82	34.16	28.25	7.83	4.99	99.00	8.66
			1.66	13.44	43.85	34.17	28.28	7.83	4.77	98.60	8.63
			1.67	13.30	43.95	34.13	28.34	7.83	4.33	98.20	8.62
			1.67	13.30	43.96	34.13	28.35	7.83	4.03	98.10	8.61
			1.67	13.32	43.94	34.14	28.34	7.83	4.55	97.90	8.59
			1.67	13.27	43.98	34.13	28.37	7.83	4.84	98.20	8.62
Little River Harbour	208	24-Sep	1.47	13.13	42.40	32.79	27.24	7.91	7.48	98.20	8.71
			2.51	13.14	42.34	32.75	27.19	7.92	7.48	98.20	8.71
			2.40	13.15	42.32	32.74	27.18	7.93	7.48	98.20	8.71
			0.87	13.14	42.21	32.65	27.10	7.92	NM	94.20	8.36
			0.95	13.11	42.17	32.59	27.07	7.92	NM	93.80	8.33
			0.95	13.10	42.16	32.58	27.06	7.92	NM	93.50	8.30
			0.95	13.10	42.14	32.56	27.05	7.92	NM	93.20	8.27
			0.96	13.10	42.12	32.55	27.04	7.92	NM	92.90	8.25
			0.96	13.09	42.12	32.54	27.03	7.92	NM	92.90	8.25
			0.96	13.08	42.11	32.52	27.03	7.92	NM	92.70	8.24
Camp Cove YSI 6600	7	24-Sep	0.95	14.67	42.81	33.14	27.57	7.69	NM	97	8.32
Clark's Harbour YSI 85	8	24-Sep	1.00	11.40	36.02	NA	31.60	NA	NA	78.50	6.95
Dennis Point YSI 6600	154	24-Sep	1.48	13.21	43.21	32.70	27.81	7.67	3.45	97.80	8.61
Falls Point YSI 85	156	24-Sep	1.00	12.40	46.60	NA	30.20	NA	NA	67.00	5.87
Wedgeport YSI 6600	6	24-Sep	0.96	14.15	38.24	NA	24.32	7.66	8.87	95.50	8.45

Appendix 3, continued.

Station and Instrument	Stn #	Date	Depth m	Temp °C	SpCon mS/cm	Cond mS/cm	Salinity psu	pH	Chla µg/L	Oxygen %	Oxygen mg/L
Morris Island YSI 6600	178	24-Sep	1.76	14.36	34.42	NA	27.66	7.71	7.48	98.10	8.45
Port La Tour YSI85	9	25-Sep	1.00	10.00	50.30	NA	32.70	NA	NA	55.40	5.43
Shelburne YSI 6600	12	25-Sep	0.70	10.28	40.31	28.98	25.66	7.79	NM	95.00	9.04
			0.70	10.28	40.32	28.98	25.66	7.79	NM	95.00	9.04
			3.01	8.40	41.91	28.62	26.67	7.76	NM	97.40	9.61
			2.69	8.33	41.97	28.60	26.70	7.75	NM	92.50	9.14
			0.65	9.93	40.93	29.14	26.07	7.76	NM	93.70	8.96
Gunning Cove YSI 6600	11	25-Sep	2.98	9.83	41.27	29.31	26.30	7.90	4.99	94.40	9.04
			2.77	9.84	41.33	29.36	26.35	7.91	4.99	94.40	9.04
			1.34	10.03	41.10	29.35	26.20	7.92	7.99	94.20	8.99
			0.69	10.00	40.25	28.72	25.60	7.93	8.36	94.30	9.04
Lower Sandy Point YSI 6600	14	25-Sep	1.07	9.66	41.96	29.66	26.78	7.87	6.67	93.00	8.91
			1.22	9.50	41.96	29.54	26.77	7.88	6.67	93.20	8.96
			3.70	9.15	42.10	29.36	26.85	7.88	6.67	93.60	9.07
			4.08	8.99	42.23	29.32	26.93	7.88	12.39	94.40	9.18
			4.32	8.97	42.27	29.33	26.96	7.88	20.96	94.40	9.17
			4.33	8.97	42.12	29.23	26.86	7.88	20.60	94.10	9.16
			3.76	8.99	42.24	29.32	26.94	7.88	14.51	93.90	9.13
			1.73	9.07	42.25	29.39	26.95	7.88	10.41	94.30	9.15
			0.83	9.51	42.02	29.59	26.82	7.88	8.36	95.50	9.18
Ingomar YSI 85		25-Sep	1.00	11	51.00	NA	33.40	NA	NA	69.8	6.04

Appendix 4: Presence of *Didemnum vexillum*, and other non-indigenous tunicate species on biofouling monitoring collectors examined *in situ* in swNS, 23-25 September 2013. Stn # = station number, ***D. v.*** = *Didemnum vexillum*, ***C. i.*** = *Ciona intestinalis*, ***B. s.*** = *Botryllus schlosseri*, ***B. v.*** = *Botrylloides violaceus*, ***S. c.*** = *Styela clava*, ***D. l.*** = *Diplosoma listerianum* and ***A. a.*** = *Ascidiella aspersa*. ND = Not detected, P = Present

Station	**Stn #**	***D. v.***	***C. i.***	***B. s.***	***B. v.***	***S. c.***	***D. l.***	***A. a.***
Yarmouth Bar	4	ND	P	P	P	ND	ND	ND
Yarmouth Yacht Club	162	ND	P	P	P	ND	ND	ND
Pinkney's Point	155	ND	P	P	P	ND	ND	ND
Wedgeport Tuna Wharf	97	ND	ND	P	P	ND	ND	ND
Wedgeport	6	ND	P	P	P	ND	ND	ND
Camp Cove	7	ND	P	P	P	ND	ND	ND
Dennis Point	154	ND	P	P	P	ND	ND	ND
Fall's Point	156	ND	P	P	P	ND	ND	ND
West Head	160	ND	P	P	P	ND	ND	ND
Clark's Harbour	8	ND	P	P	P	ND	ND	ND
Port la Tour	9	ND	P	P	P	ND	ND	ND
Ingomar	10	ND	P	P	P	ND	ND	ND
Gunning Cove	11	ND	P	P	P	ND	ND	ND
Shelburne Yacht Club	12	ND	P	P	ND	ND	ND	ND
Lower Sandy Point	13	ND	P	P	P	ND	ND	ND
Present/Total Stations		**0/15**	**14/15**	**15/15**	**14/15**	**0/15**	**0/15**	**0/15**

Appendix 5: Presence of *Didemnum vexillum*, and other non-indigenous tunicate larvae detected by molecular analysis in water samples collected in swNS, 23-25 September 2013. Stn # = station number, ***D. v.*** = *Didemnum vexillum*, ***C. i.*** = *Ciona intestinalis*, ***B. s.*** = *Botryllus schlosseri*, ***B. v.*** = *Botrylloides violaceus*, ***S. c.*** = *Styela clava*, ***D. l.*** = *Diplosoma listerianum* and ***A. a.*** = *Ascidiella aspersa*. ND = Not detected, P = Present

Station	**Stn #**	***D. v.***	***C. i.***	***B. s.***	***B. v.***	***S. c.***	***D. l.***	***A. a.***
Yarmouth Bar	4	ND	ND	P	P	ND	ND	ND
Yarmouth Yacht Club	162	ND	ND	P	ND	ND	ND	ND
Pinkney's Point	155	ND	ND	P	P	ND	ND	ND
Wedgeport	6	ND	ND	ND	P	ND	ND	ND
Camp Cove	7	ND	ND	P	P	ND	ND	ND
Dennis Point	154	ND	ND	ND	P	ND	ND	ND
Fall's Point	156	ND	ND	P	P	ND	ND	ND
Clark's Harbour	8	ND	ND	ND	P	ND	ND	ND
Port la Tour	9	ND	ND	ND	P	ND	ND	ND
Ingomar	10	ND	ND	ND	ND	ND	ND	ND
Gunning Cove	11	ND	ND	ND	ND	ND	ND	ND
Shelburne Yacht Club	12	ND	ND	ND	ND	ND	ND	ND
Lower Sandy Point	13	ND	ND	ND	P	ND	ND	ND
Present/Total Stations		**0/13**	**0/13**	**5/13**	**9/13**	**0/13**	**0/13**	**0/13**